



Epidemiological study of *Cryptosporidium parvum* parasite in some districts of Nineveh province, with referring to histopathological effects in lab mice.

Shireen H. Younis

Firas M. B. Alkhasha

**Department of Biology, Colleague of Education for Girls, University of Mosul,
Iraq**

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Abstract:

the present work was aimed to investigate the endemicity of *Cryptosporidium parvum* (The coccidian intestinal protozoan of man and other grazing animals) in some districts of Nineveh province, then evaluating the histopathological effects of the isolated parasite in inoculated mice. A total of 200 child those attended healthcare centers and diagnosed clinically to have persistent and chronic diarrhea were chose for the study. Their ages were ranged from ≤ 1 to ≥ 5 years old. Their families were practicing livestock grazing. The study were performed between October 2020 and March 2021, in 5 districts : Al- Hamdania, Bartelah, Basheqah, Fadhliah and Kokgali; respectively. Blood and stool sample were taken from each patient. Depending on direct and immunodiagnostic methods, the highest infection percentage was recorded in Kokgali district (48.1%), and the lowest was recorded in Bartelah district (27.2%). The percentage of positive cases were varied according to the performed diagnostic method: ELISA technique for stool samples (40%), ELISA technique for blood samples (36%), Zeil Neelsen stain (35%) and flotation method (30%). Infection percentage in males (44.7%) was higher than females (35.7%). Considering age groups, 3-5 years group was more likely to get cryptosporidiasis infection (56% of the positive cases). Inoculation of the parasite in lab mice showed obvious histopathological changes, especially in the epithelial brush bordered cells of intestine.



Key words: Cryptosporidiosis, *Cryptosporidium parvum*, Persistent diarrhea, Modified Zeihl Neelsen staining

Introduction: *Cryptosporidium Parvum* is an unicellular protozoan parasite that belonged to the phylum Apicomplexa. It is inhabiting the digestive tract and respiratory system of different vertebrates, including man (1) (2) (3). *C. Parvum* is one of the main causes of diarrhea in children (4). In adults, it may cause severe diarrhea and life-threatening infections in people with incompetent immunity (5)(6). Its medical and veterinary importance is depending on the strain virulence, the site of infection(7). *C. Parvum* has global distribution, particularly in third world countries were the people suffering poverty and clouded residence(6)(8). The inactive form of the parasite is called Oocyst and it is discharged with feces of the infected host (9)(10). *C. parvum* sized 4–5 μm , it is smaller than the red blood cells. The global distribution of this parasite may due to the direct life cycle and the multiple methods of transition (filth-bore infection), added to multitude reservoirs. Mortality rate in malnourishing children on account of cryptosporidiosis infection is about 9%. (11). Organ transplantation may increase the risk of cryptosporidiosis infection in adults (12). There are more than 23 species of genus *Cryptosporidium*, added to approximately 61 genetic patterns (basing on the sequence of a small subunit SSU rRNA) (13). Thus the present study was aimed to investigate the prevalence of cryptosporidiosis infection in several district in Nineveh province in children \leq five years of age, adopting different diagnostic methods, then inoculating the parasite in lab mice to study the histopathological effects in different body organs.

Materials and Methods:

Immunology study:

The current study included immunology tests using Enzyme-Linked Immunosorbent Assay ELISA on serum and stool samples taken from children under study and used this technique as a highly sensitive biochemical and immune technique that allows detection and quantification of a large number of components, based on qualitative identification of the target compound (antigen or substance to be analyzed) by antibodies associated with it, A complex anti-



antigen is detected and calibrated using an enzyme–tagged antigen, and when adding a non–colored detector gives the enzyme a color reaction in which the intensity of color is proportional to the concentration of the substance to be analyzed in the sample, which is a widely used diagnostic laboratory technique in laboratory laboratories of pathological analysis, this method is used in immunology and immune hormone analyses, one of the Advantages of the Eliza method are its ease, security and objectivity in reading results and the short time of diagnosis, especially when many samples to be examined in a short period of time are also characterized by high sensitivity up to 99%. Hundreds of diagnostic kits rely on Eliza to diagnose parasitic, viral, and bacterial diseases, including the diagnosis of parvum cryptosporidium parasite.

Blood sample collection:

200 blood samples were collected from children from whom feces samples were taken simultaneously, where blood was withdrawn from the vein using sterile medical syringes and blood samples were placed in yellow tube blood tubes (the tube contains anti–clotting material, this gel is placed in the yellow tube in a simple and undistorted manner to prevent blood clotting as well as to separate the serum) thinning the tubes with a capacity of 10 ml and leaving blood for an hour at laboratory temperature The serum was pulled by micropipette, blood samples were kept in plastic tubes, tubes were numbered and kept in frozen at temperature (-20) until use.

Collection and preparation of fecal samples:



200 fecal samples were collected from children under five years of age and both sexes with chronic and persistent diarrhea, the study included the collection of samples from domestic animal breeders such as birds and livestock. The parents of the children were given clean and numbered plastic bottles to collect samples with the age and gender of each child, the date of collection of the sample, and the residence, the samples were transferred in clean plastic containers to the graduate laboratory / Faculty of Education for Girls / Department of Life Sciences for the necessary laboratory tests, one gram of feces was mixed into (15) ml of saline solution 85% then filtered the suspension through medical gauze paper 4 layers to remove large particles and then placed in the centrifuge at a speed / 1000 minutes for 10 minutes and was kept in frozen at temperature (-20) until use.

Enzyme-linked immunosorbent Assay (ELISA) method used the direct method of investigating the *Cryptosporidium parvum* antigen (or called the *Cryptosporidium parvum*) method Sandwich method, through which the presence of antigen is measured in the blood sample, the holes in the microplate of the Eliza examination are coated with the high purity antibodies of the *Cryptosporidium parvum* parasite, which is associated with the antigen if it is detected in Stool and serum sample After incubation, the sample is washed to remove excess antibody-related antibodies and a monolithic antibody associated with HRP-conjugated, which is specifically identified as *cryptosporidium parvum* antigen, is added, followed by a second incubation period during which an immune compound is formed. (*Cryptosporidium parvum* antibody – with *cryptosporidium parvum* antibody – antibody associated with HRP if *cryptosporidium parvum* antigen is present in the test sample. By drilling with the reaction position solution and then measured in the spectroscopic reading device (Reader) where the



enzymatic activity of the antibody associated with cryptosporidium parvum proteins on the wall of each hole is proportional to the amount of Cryptosporidium parvum antigen in the test sample.

Experimental study:

The selection of laboratory animals:

40 male Swiss Albino Mice of the Balb/C strain (3–4) weeks old were used in the experimental study. (Protein, grains, and soybeans) and under the right conditions of heat ranging from (25–22) degrees Celsius, and with sufficient lighting, samples of mice feces were examined daily for a week to ensure that they are free of parasitic intestinal infections, and the samples were also examined by making a smear of feces and staining them by a Zhel– Nielsen stain to make sure they are free of *Cryptosporidium parvum* infection.

Preparation of oral doses from the stool of infected children and mice infection:

the dose of infection was prepared from *Cryptosporidium parvum* Oocyst isolated from the feces of children suffering from Cryptosporidiosis and the dose was prepared according to the method mentioned (16) .

Calculation of the infected dose of *Cryptosporidium parvum* oocyst:

The number of Oocysts found in 1 ml of the sample is calculated after the isolation and purification steps where the Hemocytometer is used to determine the dose needed for laboratory mice and the step was taken by taking a drop of the suspension on and putting it on the Hemocytometer and covering it with the slide cover and then calculating the number of Oocyst under the microscope and



the strength of enlarging X 40 and 100X in large squares and calculated the preparation of oocysts based on the following equation (13).

$$N = S / 2 \times 1000 \text{ (oocysts /ml of sample)}$$

N= number of oocysts in 1 ml of sample

S= number of oocysts in 2 cubic area

Experimental infection in laboratory animals:

The Stomach tube was used for oral injection of laboratory mice was swallowed with doses containing 2×10^3 Oocyst/animal. (17) Stools of infected mice were examined daily to confirm the appearance of Oocyst in the stool using a Zheil- Nielsen stain (M, Quilez J, Sanchez-Acedrnad F.,2000) To ensure that mice have been infected with the parasitic Oocyst, then they have been dissected and the tissue sections of the different organs have been performed and examined.

Histological tissue examination:

The tissue examination was performed by tissue sections of the organs to be examined and this is done by taking an autopsy biopsy from the affected tissue of the infected mice intestines, liver, spleen, brain, then the organ to be examined is placed in buffered formal saline solution at a concentration of 10%, cut by 5 micrometers and then we stained by Gemiza stain or toluidine blue and then examined under the microscope 40 X and X 100 to revealed the parasite and its multiple evolutionary stages on microvilli where the evolutionary stages of the parasite were seen in the form of circular blue objects of a size ranging from (1-5) micrometer depending on the evolutionary stages of the parasite's life cycle in the intestines.

Results and discussion:

Results of laboratory tests of stool samples:

Microscopic examination methods are one of the most approved methods in diagnosing parasite infection through the results of this study shows a difference in the efficiency of the microscopic diagnostic techniques used where oocysts of *Cryptosporidium parvum* were seen in stained samples by Modified Zeihl Neelsen, stain in bright red size (4–6) micrometer (18)

in circular or oval shapes and inside them, the oocysts appeared in the form of brown granules and have a dark wall and surrounded by a white halo On a blue background Figure (1)

The results of microscopic examination using the method of Zhel– Nielsen had a total infection rate of 35% and the highest infection rate was in the Kokgli region at 48.1% and the lowest infection rate was 27.2% in the Bartala region as shows in Table (1). The results of the study are agreed with the study (19) on the parasite of the oocyst using the method of Zhel– Nielsen, the results of microscopic research were 51.4% on stool samples in the chickens of Diwaniyah province .

Figure (1) *Cryptosporidium parvum* oocyst in stool sample stained with Zhel– Nielsen stain blue methylene dye as a differential dye 2000X



Table (1) Distribution of *cryptosporidium parvum* infections according to residence
(detected by Zhel- Nielsen stain)

Residence	Total cases	+ve cases	-ve cases	Rate of infection (%)
Hamdania	60	22	38	36.6
Bartela	55	15	40	27.2
Basheqa	33	12	21	36.3
Fadhliia	25	8	17	32
Kokgli	27	13	14	48.1
Total	200	70	130	35

While in the floatation method, the oocyst of *Cryptosporidium parvum* was seen in the form of circular bodies surrounded by a single thick wall and surrounded by a transparent halo with dark granules of different sizes representing the boogies (20) , however, there is some difficulty in distinguishing the oocyst of the parasite from other cells in this method, which is why the dye method was considered to be better than the method of folding the sugar solution



for the ease of distinguishing the oocyst of the parasite *Cryptosporidium parvum* through color (21). Also The current study is consistent with a study (23) which studied 780 fecal samples in five areas of Kirkuk province with an intestinal parasite infection rate of (37.56%) and the highest rate of *Cryptosporidium parvum* parasites at 16.28%, followed by *Entamoeba histolytica* at 10.12%.

Table (2) Techniques used in *Cryptosporidium parvum* parasite diagnosis with a statement of the incidence ratio of each technique

Technique used in the diagnosis	+ve cases	Rate of infection (%)
Modified Zeihl Neelsen stain	70	35
Flotation method	60	30
Eliza for stool sample	80	40
Eliza for serum sample	72	36

Through table (2) the number of cases of *Cryptosporidium parvum* in children > 5 years old of age and according to the methods used in the diagnosis and we find the highest infection rate was in the Eliza method using stool samples and reached (40%) and then Eliza serum (36)% and then the dye of Zhel- Nielsen, which reached(35)% and the method of Flotation method was the lowest percentage which is (30%) Therefore, the most important diagnostic



techniques are the Eliza method using stool samples to diagnose *Cryptosporidium parvum*.

The results are consistent with the study carried out by (22) through which he used several methods of diagnosis parasites in sheep in Nineveh province the total incidence was 26.66% and when compared the results showed the most efficient method is staining by Zhel– Nielsen stain at 13.33%, followed by floatation saline method 7.77% and iodine stain method 5.55%.

In the other hand (24) in Sudan, where recorded a 27.1% infection percentage of *cryptosporidium parvum* infection after he collected 250 stool samples from outpatients of Kosti Hospital and used Zhel– Nielsen staining method in the parasitic diagnosis and confirmed a high prevalence among children who use water sources with pipes for drinking. (25) recorded a 29.6% infection rate in Peru, and the incidence rate in people with HIV was 11.5% in Isfahan 35.9% in Mashhad (26)

Since 2000, laboratory–confirmed cases have been reported with annual infection percentage of *cryptosporidium parvum* infection in Canada estimated at 35,092 (27) The highest incidence of Cryptosporidiosis in Canada's Arctic (28) (29) showed through studies and research on parasites to study epidemiology around the world that the prevalence of parasites varies from country to region according to the geographical difference of those countries where the spread of the parasite was 1–4% in Europe and North America while 3–30% on the continents of Africa, Asia, Australia and South and Central America (30).

We conclude from the current study and previous studies that the high percentage and risk factors on the health of children in the province and the widespread spread of parasites due to many reasons including drinking of

contaminated water and raising animals close to residential areas, pollution caused by the lack of proper sanitation and the use of animal waste to fertilize vegetables and direct contact with animals are an important source of transport of parasite .

Relationship to infection with different age groups:

The current study found that the highest incidence in children was 49.4% between the ages of three to five years while the lowest incidence was in children under one year of age and 16.6% Table (3) the highest incidence was 40.90% older than two to three months, while the incidence decreased by the age of more than two to five years. The (31) study recorded a higher incidence in the 12-1 month age group than the rest of the age groups.

Table (3) Distribution of *cryptosporidium parvum* infections according to age groups and residence, (detected by ELISA for stool and serum)

Residence	Age/ Year	Total cases	Serum		Rate of infection (%)	Stool		Rate of infection (%)
			-ve cases	+ve cases		-ve cases	+ve cases	
Hamdania	>1	10	9	1	10	8	2	20
	1-3	25	19	6	24	18	7	28
	3-5	25	12	13	52	11	14	56
Bartala	>1	6	5	1	16.6	5	1	16.6
	1-3	24	18	6	25	16	8	33.3
	3-5	25	14	11	44	6	19	76
Basheqa	>1	6	5	1	16.6	5	1	16.6



	1-3	12	8	4	33.3	6	6	50
	3-5	15	9	6	40	6	9	60
Fadhli	>1	3	2	1	33.3	3	0	0
	1-3	10	7	3	30	8	2	20
	3-5	12	7	5	41.6	9	3	25
Kokgli	>1	5	4	1	20	5	0	0
	1-3	10	6	4	40	7	3	30
	3-5	12	3	9	75	7	5	41.6
Total	>1	30	25	5	16.6	26	4	13.3
	1-3	81	58	23	28.3	55	26	32
	3-5	89	45	44	49.4	39	50	56

The results of the current study vary with the findings of (32) in Dohuk and its areas, there were no cases among children and people over the age of 7 years and the infectious rate was limited only among children between the ages of 3 months and 7 years of age and from both Gender, where the incidence of children in the age group (3 months – 2 years) (42.42%) for both sexes and (39.13%) for children aged (3 years – 7 years) for both sexes and no moral difference was recorded, the number of infections was decreasing with age, so no parasitic infection was recorded in children and adults between the ages of (8 and 33) years. It also varies with the results (33) in Diwaniyah governorate for children under the age of 12, the highest incidence among children in the age group <1, at 21.52% And the lowest rate recorded in the (12–9 age group) In 2015, 10.79% of children under the less than one year were infected, followed by 10% of children aged 10 years.



The results was showed that the incidence of the parasitic Oocyst is the highest in children under five years of age in Saudi Arabia. About 30– 50% of infant and child deaths worldwide are caused by parasites and the parasite has gradually been associated with malnutrition, particularly in low– and middle– income countries (34) (35) This is due to the frequent movement of children at this age and their exposure to environmental pollution and this helps to enter many pathogens into the body through the digestive system as well as by mixing with children or contact with animals where there are reservoirs all these causes lead to the spread of parasites such as mice and rats as well as some insects help to transport parasite Oocyst. It may also be due to living in the countryside or camps that adopt animal husbandry in or near housing and which are a source of infection, in addition to the lack of attention to hygiene, health awareness and the spread of rodents. (31)

Infection rate according to Gender:

The results of the current study showed that the highest incidence was in males at 43.2% while the incidence rate in females was 30.5%. This is agreed with the results of (36) study in Hilla city, middle of Iraq, which found a 76.5% increase in the incidence of parasites in males compared to females 23.5%. The incidence rate among pupils with male parasite is 61.5% compared to females 38.5% .

Table (4) Distribution of *Cryptosporidium parvum* infection according to gender and residence,(detected by ELISA for technique stool and serum)

Residence	Sex	Total	Serum	Rate of	Stool	Rate of
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		cases	-ve cases	+ve cases	Infection (%)	-ve cases	+ve cases	Infection (%)
Hamdania	Male	30	18	12	40	16	14	46.6
	Female	30	22	8	26.6	21	9	30
Bartelah	Male	30	20	10	33.3	17	13	43.3
	Female	25	17	8	32%	15	10	40
Basheqah	Male	18	12	6	33.3	8	10	55.5
	Female	15	10	5	33.3	9	6	40
Fadhliah	Male	14	10	4	28.5	9	5	35.7
	Female	11	8	3	27.2	7	4	36.3
Kokgali	Male	13	9	4	30.7	8	5	38.4
	Female	14	9	5	35.7	9	5	35.7
Total	Male	105	69	36	34.2	58	47	44.7
	Female	95	66	29	30.5	61	34	35.7

The results are consistent with a study (23) in five areas of Kirkuk province, where the incidence rate in males was 23.55%, and the proportion of females was 21.88% and children are considered the most vulnerable group to be infected by *Cryptosporidium parvum*, especially the ages of less than six years, possibly because of the incomplete maturity of the immune system in children in addition to exposure to food and water pollutants, which leads to more infections than others.

The current study differs from the results of the study (32), where the results of the examination of the 77 stool samples, which were stained by Zhel-Nielsen staining method, showed the incidence of the parasitic parasite in 23 children and from both The incidence was very similar between males and



females, with a male infection rate of 29.72%, while among females it was 30%, and also different from the results of the study (37) Female infection rate is higher than that of males. In 2014, the incidence of female infection also increased by 19.41% compared to males of 16.96%, due to female exposure to sources of pollution more than males and malnutrition. Al-Jubouri recorded 2015 that female infection was 8.6% higher than that of males, which was 7.8%, and (38) showed a 64.52% higher incidence among females and 35% in males. 48% also differed with (39) the proportion of males 29% and females 31% in some areas of Kirkuk province.

Through the sources and results mentioned above, there is a difference in infection rates, males may be more likely to be infected because of playing outside the home and mixing with other children more. This may be because the number of male hospital reviewers is higher than that of females and may relate to certain social and environmental habits (36) and (40)

Infection rate according to Season:

d- Seasonal variation :

The most characteristic weather in Iraq, especially the northern sections, including Nineveh province, is the contrast and the great difference in the weather, for example, differences in temperatures as well as the difference in humidity, which caused multiple biological effects, so we find differences in the rates of parasite infection during the seasons of the year. In December, it was 24.2%.

In the autumn we find relative moderation in temperatures and not significant variation in relative temperature and humidity rates, while in winter a decrease in the incidence was observed due to the very low temperatures smaller

and may reach below 0 C°, especially at night, where the study of (41) showed the highest incidence in autumn and winter, reaching 14.2% and 6% respectively and the lowest infection rate in the spring at 3.4%

It is Our results consistent with the results of (23) where the highest incidence of a parasite among children of both sexes was recorded in the month of (summer) and the rate of infection of 54.54 (50%) respectively, while the lowest incidence of the parasite in the month of (January and February) where the incidence rate (18.18%) recorded (42) was the lowest incidence in the month of April 41.25% The lowest incidence in November was 27.14% in calves, who found that the incidence of infant calves in the spring months was higher than in winter. The study (43) recorded the highest incidence in March 11.9% and the lowest percentage during June 5.23% and July 5.23% on fecal samples in chicken diwaniyah province. It varies with the results recorded by (44) that the highest prevalence of Oocysts in some water sources was in the month of February and April in Basra province as shows in table (5).

Table (5) Distribution of *Cryptosporidium parvum* infection according to months of study (detected by ELSA for stool and serum)

Month	Total cases	+ve cases	-ve cases	Rate of infection (%)
October	35	22	13	62.8
November	33	12	21	36.3
December	33	8	25	24.2
January	33	9	24	27.2
February	33	13	20	39.3
March	33	16	17	48.4



Total	200	80	120	40
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The results of the current study are consistent with that of (45), who recorded highest infection rate in October and November(71.4 % and 22 % positive results respectively). On the other hands, Our results are different from the results of the (33), where the highest infection rate in July (28.89%) and the lowest infection rate in February (8.49%). These differences in the seasonal variation in spread of the parasite may due to the variation in the time in which the study performed, or may due to the imbalance of nature's conditions of heat moisture drought and the spread of parasites in winter and autumn due to rain or flows of sewage leading to pollution of the environment, herbs and water sources, but autumn spread can be caused by the emergence of insects such as flies and cockroaches it is normal for the parasite to move with insects and lead to contamination of water and food. Differences in the rate of parasitic infection are due to many factors such as the number of patient samples in the examination study, differences in the nature of the areas, the age of patients, the method of diagnosis used, life style , social and economic standards, immune status, nutritional status, personal hygiene, and the varying temperatures from year to year in the same region and from country to country. The results are consistent with a global lost study (46) reported that people who develop diarrhea after travel are more likely to seek medical attention and the highest incidence was in late autumn and early winter

Experimental study:

II– Histopathological studies :



The presence of pathological changes were generally includes the positioning of the interal stages of the parasite in the intestinal tissues of mice on the brush border of the intestinal membranes, added to the disappearance of the blood vessels and hyperplasia of the epithelium after 8–14 days of The initiation infection Furthermore, sever necrosis and hyperplasia in the epithelial cells of intestinal were observed and may spread through the gut, toward the mucosa of the stomach and the small and large intestines or may still localized In parts of the intestines affected by host immunity. Mice inoculated with *Cryptosporidium*, and the tissue sections of the intestine showed the presence of catarrhal inflammation with necrosis of the apices of intestinal villi and the presence of the parasite's reproductive phases glued to the tops of intestinal villi, as well as mucinous degeneration mucosa degeneration in the cells lining the intestinal villi and intestinal glands (**Figure 2**).

It was also observed that there were tissue–diseased vents represented by the placement of different spores on the brush edge of the intestinal slumbar epithelium with vascular congestion and hyperplasia hyperplasia in the epithelium of the slumbars and the integration of some with Epithelial cells lined with intestinal slumbars, as other passages showed the presence of tucsons of some of the tops of these fuzzes and their fall into the intestinal cavity in the form of cellular debris with hyperplasia bayer smudges and the leaching of inflammatory cells in the leptocyte Lamina propria. (**Figure 3**)

In the stomach, tissue sections showed hyperplasia hyperplasia of infected epithelial cells as well as in the infected glands with inflammatory exudate, as well as the presence of parasitic phases attached to infectious epithelial cells, where gastric pits and inside the infectious glands. Gastric glands (**Figure 4 and 5**) in the brain, microscopy showed pathological changes in vaeuolation in neurons, while

other neurons appeared to suffer from necrosis with vasogenic edema and leaching. Gliosis inflammatory cells are collected (**Figure 6**)

Histological sections of the liver showed an expansion of congestion in the central veins with vacuolated necrosis and reflux in hepatic cells with a sip of inflammatory cells, particularly lymphocytes, phagocytic and acidophills. Other sections also showed the presence of parasitic phases of hepatic patios with a dense displacement of inflammatory cells (figure 7)

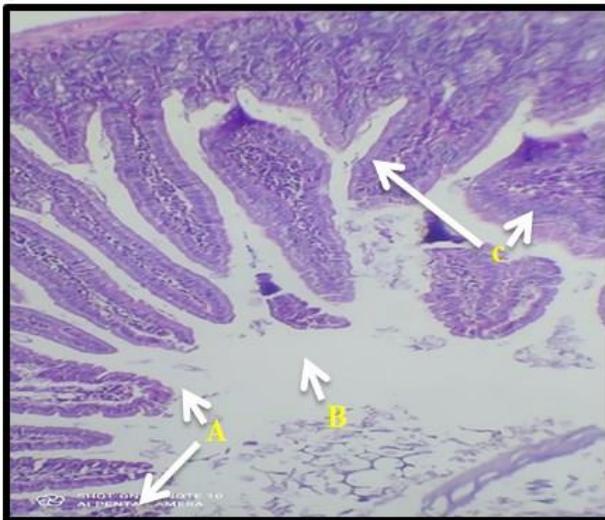


Figure 2. A cross section in the intestines of a mouse experimentally infected with *Cryptosporidium parvum* shows the presence (A) of mucous insatiabile (B) necrosis of the tops of the villi (C) presence of reproductive phases of the parasite and the leaching of inflammatory cells stained (H & E. 40 X)

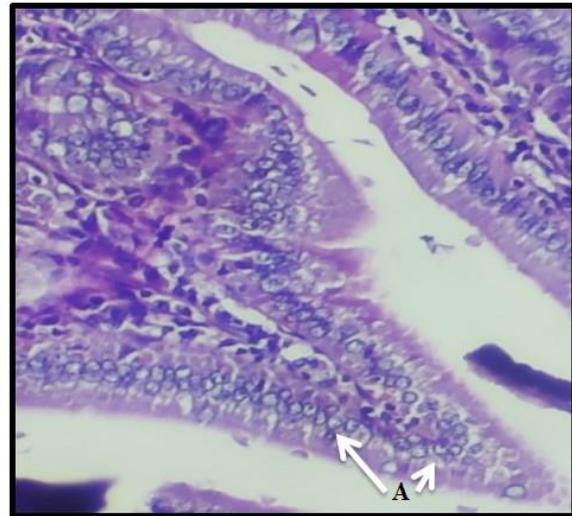


Figure 3. A cross-section in the intestines showing the presence of reproductive phases within the villi and conjoined on the edges and tops of the intestinal villi stained (H & E. 40 X)

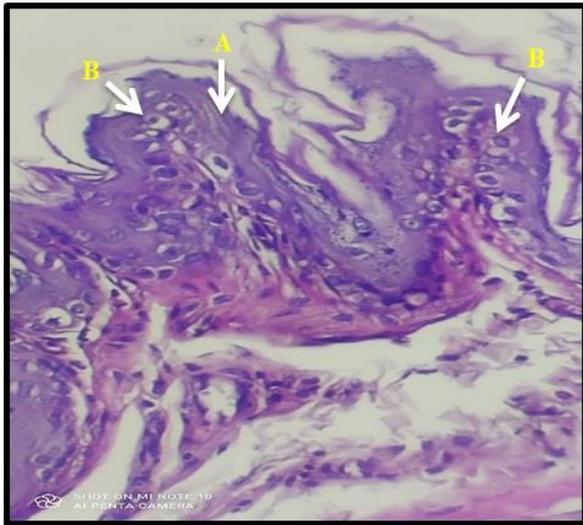


Figure 4. A cross section in the stomach of an experimentally infected mouse showing (A) hyperplasia of epithelial cells with inflammatory exudation (B) of parasitic reproductive phases within infectious epithelial cells stained (H & E. 40 X)

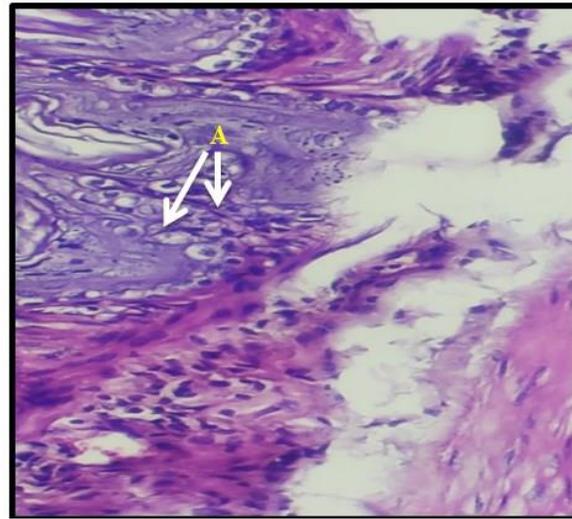


Figure 5 A cross section in the stomach of an experimentally infected mouse shows the presence of *Cryptosporidium parvum* parasite reproductive stages in the infectious mucous layer stained (H & E. 40 X)

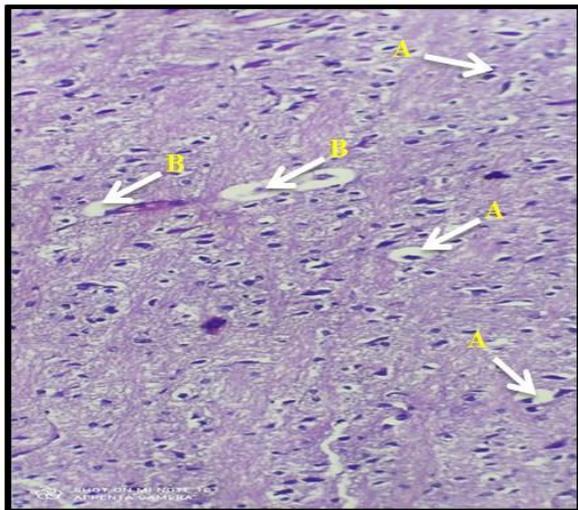


Figure 6. A cross-section of the brain of an experimentally infected mouse that

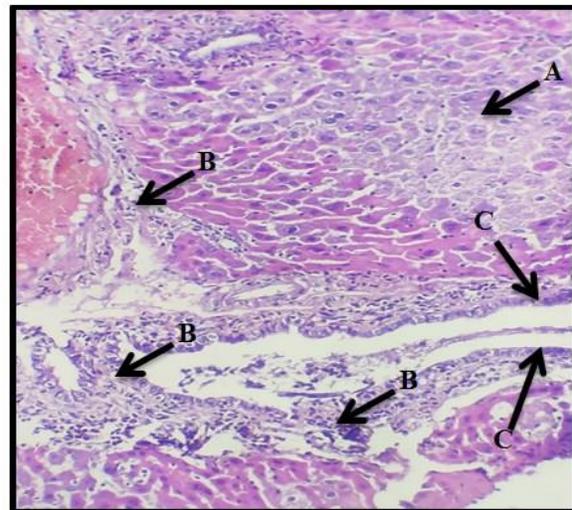


Figure 7. A cross-section of the experimentally infected mouse liver



shows (A) the spawning of neurons (B) browser section shows (A) severe for some of them as well as the throbbing of hepatic cells (B) presence of Vasogenic edema stained (reproductive stages of H & E. 40 X) *Cryptosporidium parvum* parasite inside the hepatic courtyard (C) hyperplasia of epithelial cells lining bile ducts and leaching of inflammatory cells(H & E. 40 X)

DECLARATIONS

Authors' contribution

Shireen H. Younis was involved in the development of the methodology and experimental works Firas M. B. Alkhashab contributed to data analysis and manuscript writing and performed the experimental works. Finally, after careful consideration, the final revised manuscript was approved by both authors.

Competing interests

The authors have declared no conflict of interest.

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- 1- Fayer, R.; Santin, M. & Macarisin, D. (2010). *Cryptosporidium ubiquitum* n. sp. in animals and humans. *Veterinary Parasitology*, 172(1-2): 23-32.
- 2- Xiao, L.; Fayer, R.; Ryan, U. & Upton, S. J. (2004). *Cryptosporidium* taxonomy: recent advances and implications for public health. *Clinical Microbiology Reviews*, 17(1): 72-97.
- 3- Mahmoudi, M. R.; Ongerth, J. E. & Karanis, P. (2017). *Cryptosporidium* and cryptosporidiosis: the Asian perspective. *International journal of Hygiene and Environmental Health*, 220(7): 1098-1109.
- 4- Abubakar, I.; Aliyu, S. H.; Arumugam, C.; Usman, N. K. & Hunter, P. R. (2007). Treatment of cryptosporidiosis in immunocompromised individuals: systematic review and meta-analysis. *British Journal of Clinical Pharmacology*, 63(4), 387-393.
- 5- Platts-Mills, J. A., Babji, S., Bodhidatta, L., Gratz, J., Haque, R., Havt, A., ... & Houpt, E. R. Investigators ME. (2015). Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). *Lancet Glob Health* 3: e564-e575.
- 6- Khalil, I. A., Troeger, C., Rao, P. C., Blacker, B. F., Brown, A., Brewer, T. G., ... & Mokdad, A. H. (2018). Morbidity, mortality, and long-term consequences associated with diarrhoea from *Cryptosporidium* infection in children younger than 5 years: a meta-analysis study. *The Lancet Global Health*, 6(7): e758-e768.
- 7- Kotloff, K. L.; Nataro, J. P., Blackwelder, W. C.; Nasrin, D.; Farag, T. H.; Panchalingam, S. (2013). Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the global enteric multicenter study, GEMS): a prospective, case-control study. *Lancet* 382: 209-222. doi: 10.1016/s0140-6736(13)60844-2
- 8- Kotloff, K. L.; Nasrin, D.; Blackwelder, W. C.; Wu, Y.; Farag, T.; Panchalingham, S. & Levine, M. M. (2019). The incidence, aetiology, and adverse clinical consequences of less severe diarrhoeal episodes among infants and children residing in low-income and middle-income countries: a 12-month case-control study as a follow-on to the Global Enteric Multicenter Study (GEMS). *The Lancet Global Health*, 7(5): e568-e584.



- 9- Obada, S. R. K. (2015). The effect of garlic and pomegranate extract on experimentally infected rats with *Cryptosporidium parvum* and its comparison with the drug Metranidazole. Master's thesis. College of Science . Al-Qadisiyah University.Iraq .
- 10- Liu, S.; Roellig, D. M.; Guo, Y., Li, N.; Frace, M. A.; Tang, K. & Xiao, L. (2016). Evolution of mitosome metabolism and invasion-related proteins in *Cryptosporidium*. *BMC genomics*, 17(1): 1-16.
- 11- Liu, A.; Gong, B.; Liu, X.; Shen, Y.; Wu, Y.; Zhang, W. & Cao, J. (2020). Aretrospective epidemiological analysis of human *Cryptosporidium* infection in China during the past three decades (1987-2018). *PLoS neglected tropical diseases*, 14(3): e0008146
- 12- Lanternier, F.; Amazzough, K.; Favennec, L.; Mamzer-Bruneel, M. F.; Abdoul, H.; Turret, J. & Transplant *Cryptosporidium* Study Group. (2017). *Cryptosporidium* spp. infection in solid organ transplantation: the nationwide "TRANSCRYPTO" study. *Transplantation*, 101(4): 826.
- 13- Xiao, L.; Fayer, R.; Ryan, U. & Upton, S. J. (2004). *Cryptosporidium* taxonomy: recent advances and implications for public health. *Clinical Microbiology Reviews*, 17(1): 72-97.
- 14- Ryan, U. M.; Power, M. & Xiao, L. (2008). *Cryptosporidium fayeri* n. sp.(Apicomplexa: Cryptosporidiidae) from the Red Kangaroo (*Macropus rufus*). *Journal of Eukaryotic Microbiology*, 55(1), 22-26.
- 15- Murray, C. K.; Beckius, M. L.; Green, J. A. & Hospenthal, D. R. (2005). Use of chromogenic medium in the isolation of yeasts from clinical specimens. *Journal of Medical Microbiology*, 54(10): 981-985.
- 16- Harp, J. A.; Fayer, R.; Pesch, B. A. & Jackson, G. J. (1996). Effect of pasteurization on infectivity of *Cryptosporidium parvum* oocysts in water and milk. *Applied and Environmental Microbiology*, 62(8), 2866-2868.
- 17- Nesterenko, M. V.; Woods, K. & Upton, S. J. (1999). Receptor/ligand interactions between *Cryptosporidium parvum* and the surface of the host cell. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 1454(2): 165-173.
- 18- Potters, I. & Van Esbroeck, M. (2010). Negative staining technique of Heine for the detection of *Cryptosporidium* spp.: a fast and simple screening technique. *The Open Parasitology Journal*, 4(1).



- 19- Jarad, N. I. (2020). Molecular detection of *Cryptosporidium parvum* in chicken in Al-Diwaniya province. *Iraqi Journal of Veterinary Sciences*, 34(2): 441-445.
- 20- Bakr, M. H. H. (2005). An experimental and transitional epidemiological and immunological study of *Cryptosporidium* in Nineveh Governorate. PhD thesis, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.
- 21- Darwish, M. K. and Ahmed H. M. (2016). Detecting hidden spores in flocks of broiler chickens in the Syrian coast, *Al-Baath University Journal* , 38(12) .
- 22- Abdullah, I. A. (2005). Prevalence of *Cryptosporidium* in sheep in different areas of Nineveh Governorate, *Al-Rafidain Journal of Science*, 16(7) 93-101.
- 23- Salman, Y. J.; Sadek, W. S. & Rasheed, Z. K. (2015). Prevalence of *Cryptosporidium parvum* among Iraqi displaced people in Kirkuk city using direct microscopy, flotation technique and ELISA-copro antigen test. *Int. J. Curr. Microbiol. App. Sci*, 4(11): 559-572.
- 24- Tamomh, A. G.; Agha, A. M.; Elamin, E.; Suliman, M. A.; Elmadani, M.; Omara, A. B. & Musa, S. A. (2021). Prevalence of cryptosporidiosis among children with diarrhoea under five years admitted to Kosti teaching hospital, Kosti city, Sudan. *BMC Infectious Diseases*, 21(1): 1-6.
- 25- Cama, V. A.; Bern, C.; Roberts, J.; Cabrera, L.; Sterling, C. R.; Ortega, Y. & Xiao, L. (2008). *Cryptosporidium* specie and subtypes and clinical manifestations in children, Peru. *Emerging Infectious Diseases*, 14(10): 1567.
- 26- Izadi, S.; Mohaghegh, M. A.; Ghayour-Najafabadi, Z.; Azami, M.; Mirzaei, F.; Namdar, F. & Hejazi, S. H. (2020). Frequency and Molecular Identification of *Cryptosporidium* species among immunocompromised patients referred to hospitals, central Iran, 2015-16. *Iranian Journal of Parasitology*, 15(1): 31.
- 27- Murray, R.; Tataryn, J.; Pintar, K. & Thomas, M. K. (2017). Estimates of the burden of illness for eight enteric pathogens associated with animal contact in Canada. *Epidemiology & Infection*, 145(16): 3413-3423.
- 28- Iqbal, A.; Goldfarb, D. M.; Slinger, R. & Dixon, B. R. (2015). Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia duodenalis* in diarrhoeic patients in the Qikiqtani Region, Nunavut, Canada. *International Journal of Circumpolar Health*, 74(1): 27713.



- 29- Ayres Hutter, J.; Dion, R.; Irace-Cima, A.; Fiset, M.; Guy, R.; Dixon, B. & Thivierge, K. (2020). *Cryptosporidium* spp.: Human incidence, molecular characterization and associated exposures in Québec, Canada (2016–2017). *Plos One*, 15(2): e0228986.
- 30- Water, S.& World Health Organization (2009). Risk assessment of *Cryptosporidium* in drinking water (No. WHO/HSE/WSH/09.04). World Health Organization.
- 31- Makhlaf, M. M. (2008). Study of the prevalence of the parasite *Cryptosporidium parvum* in children under five years of age in the city of Ramadi and its suburbs, Anbar University Journal of Pure Sciences, 2(2).
- 32- AL Khalil, M. I. K. I. (2017). An epidemiological and molecular study to diagnose the *Cryptosporidium* sp. in some areas of Dohuk governorate. Master's Thesis, Department of Life Sciences , College of Science , University of Mosul.Iraq.
- 33- Al-Shabani, R. H. (2016). An epidemiological study of the *Cryptosporidium parvum* parasite of children in Al-Diwaniyah governorate. Master's Thesis, Department of Life Sciences, College of Science University of Al-Qadisiyah, Al-Qadisiyah Journal for Pure Sciences, 21 (1).
- 34- Ehsan, M. A.; Akter, M.; Ahammed, M., Ali, M. A. & Ahmed, M. U. (2016). Prevalence and clinical importance of *Cryptosporidium* and *Giardia* in human and animals. *Bangladesh Journal of Veterinary Medicine*, 14(2): 109–122.
- 35- Sannella, A. R.; Suputtamongkol, Y.; Wongsawat, E. & Cacciò, S. M. (2019). A retrospective molecular study of *Cryptosporidium* species and genotypes in HIV–infected patients from Thailand. *Parasites & vectors*, 12(1):1–6.
- 36- Maaleh, S. F. A. M. (2008). Epidemiological study of *Cryptosporidium parvum* in Al-Hilla city. Master's Thesis, College of Education for Girls, University of Kufa, Iraq.
- 37- Ali, J. K. and Al-Amri, A. M. and Abdel-Taif, B. M. (2001): The prevalence of *Cryptosporidium* parasite among infants in Babel province, Al-Tanaqi Journal of Medical Research 97: 22–27.
- 38- Ur-Rahman, K. Al-Amoudi, A.; Badreddine, S.; Al-Shehri, A. M.; Kanaan, H. & Al-Ghamdi, S. M. (2007). *Cryptosporidiosis* in a renal transplant patient treated with paromomycin. *Annals of Saudi medicine*, 27(5): 373–374.



- 39- Ali, A. A. (2007). Epidemiological study of Cryptosporidiosis in some areas of Kirkuk. Technical Journal, Kirkuk University,20(1): A19-A24
- 40- Khan, A.; Shaik, J. S. & Grigg, M. E. (2018). Genomics and molecular epidemiology of Cryptosporidium species. Acta Tropica, 184: 1-14
- 41- Al-Jubouri, S. A.Mohammed Sh. M. (2015). Epidemiology of cryptosporidiosis among patients and patients in Samarra general hospital, Tikrit Journal of Pure Sciences,(3)20. ISSN:1813-1662
- 42- Khalil, M. M. and Al-Zubaidi, M. T. S. and Kazem, F. S. (2011). A study in the epidemiology of the Cryptosporidium parasite and a comparison of the efficiency of the ELISA method with some traditional methods in diagnosing infection in calves, Iraqi Veterinary Medical Journal 35(2): 145-155.
- 43- Jarad, N. I. (2020). Molecular detection of Cryptosporidium parvum in chicken in Al-Diwaniya province. Iraqi Journal of Veterinary Sciences, 34(2): 441-445.
- 44- Awad, A. H. H. and Nasser, S. K. (2014). Study of the spread of Cryptosporidium sp sacs in some Basra Governorate water sources, Thi-Qar Science Journal, Volume 4 (3): 9-12.
- 45- Al-Jarjari, S. A. (2001) Epidemiological study of Cryptosporidiosis and the role of water in the spread of the parasite, Master's thesis, College of Education for Girls, Tikrit University.Iraq.
- 46- Guy, R. A.; Yanta, C. A.; Muchaal, P. K.; Rankin, M. A.; Thivierge, K.; Lau, R. & Boggild, A. K. (2021). Molecular characterization of Cryptosporidium isolates from humans in Ontario, Canada. Parasites & Vectors, 14(1): 1-14.